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(54) Title: ANTIVIRAL AGENT COMPRISING ANTISENSE OLIGONUCLEOTIDES COMPLEMENTARY TO THE BHRF1 GENE OF EPSTEIN-BARR VIRUS

(57) Abstract

The invention provides antisense oligonucleotides to the BHRF1 gene of Epstein-Barr virus (EBV), and the use of such oligonucleotides in methods of treatment of EBV infections.

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ANTIVIRAL AGENT COMPRISING ANTISENSE OLIGONUCLEOTIDES COMPLEMENTARY TO THE BHRF1 GENE OF EPSTEIN-BARR VIRUS.

The present invention relates to the use of antisense oligonucleotides in the treatment of Epstein-Barr Virus (EBV) infections, eg. EBV related tumors such as lymphomas.

The Epstein-Barr Virus is a human herpes virus which co-evolved with our species and frequently establishes a persistent asymptomatic infection of the circulating B-lymphocyte pool. The mechanism of virus persistence is not understood but given the limited life span of most B cells in vivo it seems most likely that EBV infected cells must gain access to the long-lived memory B cell pool. (Klein 1989; Gregory C et al 1991). EBV is the causative agent of infectious mononucleosis and is associated with endemic African Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, AIDS associated lymphoma and recently has been found in association with Hodgkin's disease. (Zur Hausen et al 1970; Cleary M et al 1984; Herbst H et al 1991).

EBV exists in two stages, the latent and lytic cycles. During the latent cycle the EBV genome is incorporated with the host DNA and is non replicative whilst in the lytic cycle viral progeny are produced and this may result in lysis of the host cell.

Recently the EBV genome has been identified in Hodgkin's disease cells. This has been made possible by developments in the techniques of molecular biology enabling detection of rare copies of mRNA and DNA. This has been necessary as the malignant cells in Hodgkin's disease generally represent a small proportion of a given tumour biopsy with the remainder being an infiltrate of reactive cells. The EBV genome (and its gene products) are most consistently associated with the nodular sclerosing and mixed cellularity subtypes of Hodgkin's disease.

Hodgkin's disease can be sub-divided into four groups on the basis of morphological criteria. These are, lymphocyte predominant, nodular sclerosing, mixed cellularity and lymphocyte depleted. This sub-division translates into a different prognosis for each group. Overall the survival of patients with Hodgkin's disease is 80%. There remains a group of patients who are incurable either due to advanced or resistant disease. The curative treatment options range from radiotherapy (for localised disease) to cytotoxic chemotherapy with or without radiotherapy. A group of patients will demonstrate a pattern of persistent relapse and currently their best chance of cure lies with the high dose chemotherapy plus autologous bone marrow transplant. All therapies to date are nonspecific and are associated with toxicity. There is therefore a requirement for novel therapeutic options.

The EBV-induced early antigen complex (Henle et al 1971) is composed of restricted and diffuse components. BHRFI is an early antigen (restricted) protein and is encoded by the EBV DNA BamH1 restriction fragment 1 (BHRFI). (Pearson et al 1987) BHRFI is a 17-KD protein, expressed at the interface between the latent and lytic cycles. In vitro studies indicate it is synthesised during the g1 phase of the cell cycle before the initiation of DNA synthesis. (Kocache et al 1990).

It has been found that BHRFI has 40% homology with the bcl2 gene which is deregulated in at least 90% of cases of follicular non-Hodgkin's lymphoma. Bcl2 is expressed at the inner mitochondrial membrane and deregulation appears to extend cell survival by interrupting programmed cell death (apoptosis) (Hockenbery et al 1990) BHRFI and bcl2 therefore may have functional equivalence.

We have now surprisingly found that BHRFI is localised to the mitochondria and that expression is found only in cells in

the lytic cycle (i.e. producing intact virus) and that most of these cells are undergoing apoptosis.

While we do not wish to be bound by any one particular theory, we believe that EBV expresses BHRFI to extend survival of the host cell so as to maximise the yield of virus.

The sequence listing which follows the examples shows the sequence of the EBV genome in the region of the BHRFI gene together with a translation of the BHRFI gene. The sequence shown corresponds to nucleotides 54307 to 55020 of the EBV genome in Genbank release 26.0.

The present invention thus relates to a method of reducing the expression of BHRFI in cells infected with EBV. In particular, the present invention provides an antisense oligonucleotide which is selectively hybridizable to the BHRFI gene, compositions comprising such oligonucleotides including compositions of the oligonucleotides in admixture with a pharmaceutically acceptable carrier.

The invention further provides such an antisense oligonucleotide for use in a method of treatment or therapy of EBV infection of the human or animal body.

The invention also relates to a method of treatment of a human patient infected with EBV and in need of therapy which comprises administering to the patient an effective amount of an antisense oligonucleotide to the BHRFI gene.

Tumours containing the EBV genome are found in patients suffering from, Hodgkin's disease Burkitt;s lymphoma, undifferentiated nasopharyngeal carcinoma, AIDs related lymphoma and angioimmunoblastic lymphadenopathy and thus the above method of treatment may be used to treat patients suffering from such conditions. Infectious mononucleosis is

also associated with EBV.

The term "BHRFI gene" includes the entire transcribed region of this gene and also those 3′ and 5′ regions involved in the regulation of gene expression. The wild-type EBV produces a mRNA encoding the BHRFI gene product of about 1.9 kb in size. This RNA is encoded by nucleotides 53804 to about 55700 of the EBV geome in Genbank release 26.0. Methods for determining regulatory regions, eg. promoter regions are well known in the art.

Although oligonucleotides according to the invention will generally comprise a sequence which is exactly complementary to a part of the BHRFI gene, for example the sequence of Figure 1 (or the corresponding sequence of any other wild-type strain of EBV), some variation of the sequence may be desirable or possible, provided that such variation allows the oligonucleotides to remain selectively hybridizable to their target region of the BHRFI gene. By "selectively hybridizable" it is meant that the sequence of the oligonucleotide will be more similar to the complement of its intended target in the EBV genome than it is to any other nucleic acid sequence in the environment in which it is used. Usually, such an environment will contain the entire EBV genome, human genomic DNA, and the RNA expressed from both genomes. Occasionally, the environment may include other human viruses, eg HIV-1 or HIV-2.

It will thus be understood that reference herein to oligonucleotides according to the invention encompasses such selectively hybridizable variants. Generally, the oligonucleotide will not contain more than 1 to 5, eg 2, 3 or 4 differences between its sequence and that of the complement of the corresponding portion of the BHRFI gene against which it is directed.

Antisense oligonucleotides are short fragments of DNA or RNA

(oligonucleotides) which by virtue of their nucleotide sequence are designed to hybridise to DNA or RNA targets and thereby block transcription or translation of a specific gene. If expression of the target gene expression is essential for survival of the malignant cell population then gene blockade would result in cell death (reviewed in Helene 1990).

From the foregoing paragraph, it is to be understood that an olignucleotide designed to target the RNA transcribed from a gene will be antisense to the plus strand of the EBV genome (ie. the plus strand as shown in SEQ ID. No. 1). However, an oligonucleotide designed to be antisense to the DNA of EBV may be antisense to either strand of the double stranded EBV genome. For example, translation or transcription factors which recognise specific sequences of either the plus or minus strands of EBV could be targeted by supplying oligonucleotide comprising such sequences which will then compete for such factors with the native EBV.

It is however generally preferred that an oligonucleotide of the invention will be complementary to at least a portion of the mRNA transcript of the EBV BHRFI gene.

For in vivo activity antisense oligonucleotides will require modifications in the molecules backbone and possibly additions at either the 5′ or 3′, or both, ends of the molecule. This is to (1) achieve resistance to degradation by nucleases such as DNAases, (2) enhance the potency of the molecule and (3) to enhance uptake of the oligonucleotide by cells. This is an area of intense research and development. Currently the technology exists to produce sufficient quantities of modified oligonucleotides for therapeutic use (Biosystems Reporter 1991). A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3′ and/or 5′

ends of the molecule. Further modified oligonucleotides within the scope of the invention include polyamide nucleic acids. For the purposes of the present invention, it is to be understood that references to oligonucleotides herein include oligonucleotides modified by any method available in the art in order to enhance their in vivo activity or lifespan.

Additions to the 5′ end include mono-, di- or tri-phosphate moieties.

Further, it will be clear to those of skill in the art that although specific oligonucleotides described herein are represented in standard notation, ie., by the letters a, c, g, t (or u) to indicate the base of the nucleotide, written in a 5′ to 3′ direction (unless specified to the contrary), these sequences may be modified as described above. In addition, other types of oligonucleotides which contain nucleotide residues which are N-glycosides or C-glycosides of purine or pyrimidine bases, or modified purine or pyrimidine bases, are included within the scope of the invention and are encompassed by the term oligonucleotide. Examples of modified nucleotides include 2′-deoxy-2-aminoadenosine, 2′-deoxy-inosine and 2′-deoxy-xanthosine. Further Examples of modified oligonucleotides which may be used in the present invention are disclosed in PCT/US91/03680.

Thus, oligonucleotides according to the present invention will typically be from 6 to 30 bases in length, eg. from 8 to 25 or 15 to 25, preferably from 10 to 20 or 21, eg. 14, 15, 16, 17, 18 or 19 bases.

The oligonucleotides may be directed against any part of the BHRFI gene. One region of the gene of interest is the 5′ translated region, starting at or within a few nucleotides (eg. 3 to 12) of the initiation "atg" codon. However, antisense oligonucleotides to other regions of the gene may

be used, eg. from the 5′ untranslated region, anywhere in the translated region including the 3′ translated region or the 3′ untranslated region.

We have found that in vitro that oligonucleotides may be used effectively at a concentration of about 5μM, eg from 500nM to 500μM, eg. from 1μM to 50μM. For in vivo use the amount of oligonucleotide which may be used will ultimately be determined by a physician, taking into account the requirements of a patient, although it is expected that it will be necessary and desirable to deliver concentrations of oligonucleotides in the order of those mentioned above to the environment of infected cells.

Cells of the human body infected with EBV may be treated in vitro and reintroduced into the body. This is a technique which finds application in, for example, autologous bone marrow transplants. Accordingly, the invention provides a method for the treatment of EBV-induced proliferative diseases of white blood cells such as Hodgkin's disease which comprises removing the bone marrow of a patient in need of such treatment, treating the marrow with an oligonucleotide according to the invention and reimplanting the marrow into the patient following treatment of the patient to destroy proliferating tumour cells which remain in the patient.

Oligonucleotides according to the invention may be a single specific sequence or a mixture of different sequences.

Oligonucleotides according to the invention may be mixed with carriers or diluents suitable for delivery to a patient. Oligonucleotides may be formulated as injectable compositions, or in solid tablet form for oral administration. Other formulations, eg, for nasal, topical, rectal or vaginal administration may be used. The oligonucleotides may be formulated in unit dosage forms for single or multiple daily administration. Injectable

compositions may be formulated to be delivered as a continuous infusion.

Further, oligonucleotides according to the invention may be formulated to enhance or target their delivery. They may be encapsulated in liposomes or attached to antibodies (or fragments thereof) suitable to target cells with EBV infection. BHRFI is potentially an ideal target for the antisense approach since this gene would be unique to EBV infected cells and furthermore these cells often display epitomes which may enable antibody directed targeting of the oligonucleotides to the malignant cell population. The oligonucleotides may also be linked to other carriers, eg proteins or other nucleic acids.

One antisense oligonucleotide according to the invention is a 17mer directed against the 5′ translated region of BHRFI. It has the following sequence: 5′ ct tgt tga ata ggc cat 3′. Other oligonucleotides according to the invention include oligonucleotides encompassed by this sequence, eg:

5′ t tgt tga ata ggc cat 3′;
5′ tgt tga ata ggc cat 3′; and
5′ gt gta ata ggc cat 3′.

The following examples illustrate the invention.

Example 1.

Cell Line and Culture Conditions

To explore the function BHRFI we have examined an EBV-genome positive cell line, B95.8, using low temperature embedding immunoloectron microscopy.

B95.8 was cultured in serum free medium (RPMI 1641). Under these conditions expression of BHRFI is enhanced. On day 0 cell in log phase growth were seeded at 2 x 10^5/ml in total volume of 1ml in 24 well microtitre plates. Oligonucleotides

(sense and antisense) were added at a concentration of 5μM.

Oligonucleotides

Phosphodiester oligonucleotide (17 mer) sequence antisense to the start codon and the 14 bases 3′ was assayed with the sense sequence serving as the control. These oligonucleotides were also assayed on an EBV negative cell line.

sense 5′ atg gcc tat tca aca ag 3′

(start codon underlined)

antisense 5′ ct tgt tga ata ggc cat 3′

Cells were counted on days 1, 2 and 3 using a haemocytometer.

Results

Three experiments were performed. On day 3 the surviving fraction of cells cultured with the antisense oligonucleotide was 50% of that of the controls (either no oligonucleotide or sense oligonucleotide). The antisense oligonucleotide was inactive in the EBV negative cell line.

Example 2.

Phosphorothioate oligonucleotides were made with the following sequences:

Antisense: 3′ ctcgttctaccggataagtt 5′

Sense: 5′ gagcaagatggcctattcaa 3′

16.5um of the oligonucleotides were added to cells and the cells cultured as described in Example 1. Figure 1 shows the fraction of cells surviving over a four day period compared to untreated control cells (surviving fraction = 1).

References

Biosystems Reporter May 1991 1-2

Cleary. M et al 1984 Lancet 489-493

Gregory. C. et al 1991 Nature 349 612-614

Helene. C et al 1990 Biochemical et Biophysical Acta 1049 99-125

Herbst H et al 1991 Proc. Natl. Acad. Sci. USA 88 4766-4770

Henle. W et al 1971 Int. J Cancer 8 272-278

Hockenberry D et al 1990 Nature 348 334-338

Klein G 1989 Cell 58 5-8

Kocache M et al 1990 Intervirology 31 1-13

Pallesen G et al 1991 The Lancet 337 320-322

Pearson GR et al 1987 Virology 160 151-161

Uhlmann et al 1990 Chemical Reviews 90 544-584

Zur Hausen H et al 1970 Nature 228 1056-1058

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 720 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Epstein-Barr virus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 76..648
(D) OTHER INFORMATION: /product= "BHRFI gene product"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..720
(D) OTHER INFORMATION: /note= "Corresponds to 54301..55020 of EBV sequence of GenBank release 26.0"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACGTGGTGCT AACTTGAATT TTTTGGTTTT CTAGTTCCCT CTTAATTACA TTTGTGCCAG      60

ATCTTGTAGA GCAAG ATG GCC TAT TCA ACA AGG GAG ATA CTG TTA GCC CTG      111
                 Met Ala Tyr Ser Thr Arg Glu Ile Leu Leu Ala Leu
                   1               5                  10

TGT ATA CGG GAC AGT CGT GTG CAT GGA AAT GGT ACC CTG CAT CCT GTG      159
Cys Ile Arg Asp Ser Arg Val His Gly Asn Gly Thr Leu His Pro Val
         15                  20                  25

TTG GAG CTA GCA GCA AGA GAA ACA CCT CTC CGC CTT TCG CCA GAG GAC      207
Leu Glu Leu Ala Ala Arg Glu Thr Pro Leu Arg Leu Ser Pro Glu Asp
     30                  35                  40

ACT GTA GTT CTG CGT TAT CAT GTG TTG CTT GAG GAG ATA ATT GAA CGA      255
Thr Val Val Leu Arg Tyr His Val Leu Leu Glu Glu Ile Ile Glu Arg
 45                  50                  55                  60

AAT TCA GAG ACA TTT ACA GAA ACT TGG AAC AGA TTT ATA ACA CAC ACC      303
Asn Ser Glu Thr Phe Thr Glu Thr Trp Asn Arg Phe Ile Thr His Thr
                 65                  70                  75

GAA CAT GTG GAT CTG GAT TTT AAC TCA GTA TTT TTA GAG ATA TTT CAC      351
Glu His Val Asp Leu Asp Phe Asn Ser Val Phe Leu Glu Ile Phe His
             80                  85                  90

CGT GGA GAC CCA AGC CTT GGG CGC GCG TTG GCC TGG ATG GCC TGG TGC      399
Arg Gly Asp Pro Ser Leu Gly Arg Ala Leu Ala Trp Met Ala Trp Cys
         95                  100                 105

ATG CAT GCC TGC AGG ACA TTG TGT TGT AAC CAG TCT ACT CCT TAC TAT      447
Met His Ala Cys Arg Thr Leu Cys Cys Asn Gln Ser Thr Pro Tyr Tyr
     110                 115                 120

GTT GTG GAC CTG TCA GTT CGT GGG ATG TTA GAA GCC AGC GAA GGC CTG      495
Val Val Asp Leu Ser Val Arg Gly Met Leu Glu Ala Ser Glu Gly Leu
 125                 130                 135                 140
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GAT GGT TGG ATT CAT CAA CAG GGC GGC TGG TCT ACA TTA ATT GAA GAC        543
Asp Gly Trp Ile His Gln Gln Gly Gly Trp Ser Thr Leu Ile Glu Asp
                145                 150                 155

AAC ATT CCT GGA TCC AGA AGG TTT AGC TGG ACT TTG TTT CTT GCT GGA        591
Asn Ile Pro Gly Ser Arg Arg Phe Ser Trp Thr Leu Phe Leu Ala Gly
            160                 165                 170

CTG ACT TTG AGT CTG TTA GTT ATA TGT AGT TAT TTA TTT ATC TCC AGA        639
Leu Thr Leu Ser Leu Leu Val Ile Cys Ser Tyr Leu Phe Ile Ser Arg
        175                 180                 185

GGA AGA CAC TAATCTATAC ATTTTCTCAG CACTTTATAT GAATCAGGGT                688
Gly Arg His
    190

CATTGGGCCT GCGGGGAACT GAGCCAGTAG GA                                    720
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Tyr Ser Thr Arg Glu Ile Leu Leu Ala Leu Cys Ile Arg Asp
  1               5                  10                  15

Ser Arg Val His Gly Asn Gly Thr Leu His Pro Val Leu Glu Leu Ala
            20                  25                  30
Ala Arg Glu Thr Pro Leu Arg Leu Ser Pro Glu Asp Thr Val Val Leu
        35                  40                  45

Arg Tyr His Val Leu Leu Glu Glu Ile Ile Glu Arg Asn Ser Glu Thr
    50                  55                  60

Phe Thr Glu Thr Trp Asn Arg Phe Ile Thr His Thr Glu His Val Asp
65                  70                  75                  80

Leu Asp Phe Asn Ser Val Phe Leu Glu Ile Phe His Arg Gly Asp Pro
                85                  90                  95

Ser Leu Gly Arg Ala Leu Ala Trp Met Ala Trp Cys Met His Ala Cys
            100                 105                 110

Arg Thr Leu Cys Cys Asn Gln Ser Thr Pro Tyr Tyr Val Val Asp Leu
        115                 120                 125

Ser Val Arg Gly Met Leu Glu Ala Ser Glu Gly Leu Asp Gly Trp Ile
    130                 135                 140

His Gln Gln Gly Gly Trp Ser Thr Leu Ile Glu Asp Asn Ile Pro Gly
145                 150                 155                 160

Ser Arg Arg Phe Ser Trp Thr Leu Phe Leu Ala Gly Leu Thr Leu Ser
                165                 170                 175

Leu Leu Val Ile Cys Ser Tyr Leu Phe Ile Ser Arg Gly Arg His
            180                 185                 190
```

CLAIMS

1. An antisense oligonucleotide which is selectively hybridizable to the BHRFI gene.

2. An oligonucleotide according to claim 1 which is to either strand of the sequence of SEQ ID. No. 1.

3. An oligonucleotide according to any one of the preceding claims which is directed to the translated region of the BHRFI gene.

4. An oligonucleotide according to any one of the preceding claims which is directed against the coding strand of the sequence of SEQ ID No.1.

5. An oligonucleotide according to any one of the preceding claims which is from 10 to 25 nucleotides in length.

6. An oligonucleotide according to claim 5 which is from 15 to 20 nucleotides in length.

7. An oligonucleotide according to any one of the preceding claims which is a DNA oligonucleotide.

8. An oligonucleotide according to any one of the preceding claims which has a modified backbone and/or 5′ end and/or 3′ end.

9. An oligonucleotide according to claim 8 which has a triphosphate moiety at its 5′ end.

10. An oligonucleotide according to any one of the preceding claims which has the following sequence:

5′ cttgttgaataggccat 3′; or

5′ aacttatccggtagaacgag 3′.

11. A composition comprising the oligonucleotide of any one of the preceding claims in admixture with a pharmaceutically acceptable carrier.

12. An antisense oligonucleotide as defined in any one of claims 1 to 11 for use in a method of treatment or therapy of EBV infection of the human or animal body.

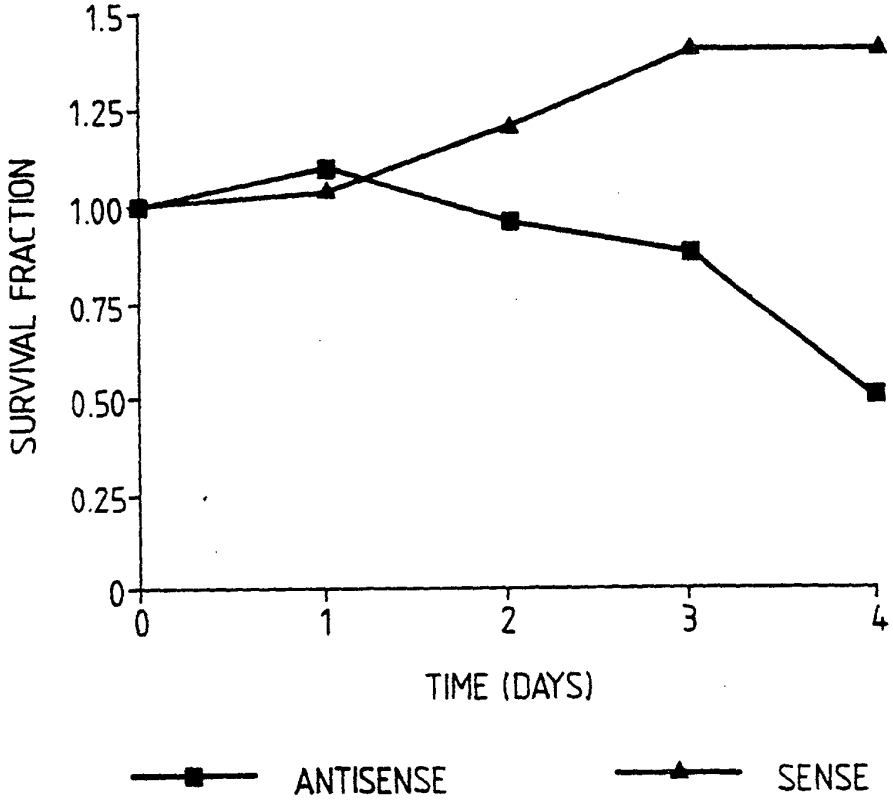
13. An antisense oligonucleotide as defined in any one of claims 1 to 11 for use in a method of treatment of EBV infected cells in autologous bone marrow transplants of the human or animal body.

14. A method of treatment of an EBV infection of a patient which comprises administering to a patient in need of treatment an effective amount of an antisense oligonucleotide to the BHRFI gene.

15. A method according to claim 14 wherein the patient is suffering from one or more conditions from the group consiting of infectious mononucleosis, Hodgkin's disease Burkitt;s lymphoma, undifferentiated nasopharyngeal carcinoma, AIDs related lymphoma and angioimmunoblastic lymphadenopathy.

16. A method for the treatment of EBV-induced proliferative diseases which comprises removing the bone marrow of a patient in need of such treatment, treating the marrow with an oligonucleotide according to the invention and reimplanting the marrow into the patient following treatment of the patient to destroy proliferating tumour cells.

Fig.1.


1.5
1.25
1.00
0.75
0.50
0.25
0
SURVIVAL FRACTION
0
1
2
3
4
TIME (DAYS)
ANTISENSE
SENSE

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12Q1/70; C07H21/02; C07H21/04; A61K31/70

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12Q ; C07H ; C12N ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 85, June 1988, WASHINGTON US pages 3678 - 3682 P.J. AUSTIN ET AL. see abstract see page 3680, right column, line 29 - page 3681, left column, line 8 ---	1,3-7, 12,13
A	VIROLOGY vol. 160, no. 1, September 1987, NEW YORK, USA pages 151 - 161 G.R. PEARSON ET AL. cited in the application see abstract see " introduction " and " discussion " --- -/--	1

[10] Special categories of cited documents :

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 MARCH 1993	24. 03. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LUZZATTO E.R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 112 811 (ISIS PHARMACEUTICALS, INC.) 5 September 1991 see page 5, line 16 - page 6, line 18 see page 18, line 22 - page 20, line 14; claims	1,2
P,X	WO,A,9 204 903 (THE UNITED STATES OF AMERICA) 2 April 1992 see the whole document, especially fig. 2 and claims	1-3

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9202256 SA 67349

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9112811	05-09-91	AU-A- 7747091	18-09-91
		EP-A- 0517859	16-12-92
WO-A-9204903	02-04-92	AU-A- 8872191	15-04-92